Research

Maternal Plasma Perfluoroalkyl Substances and Miscarriage: A Nested Case–Control Study in the Danish National Birth Cohort

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BACKGROUND: Per- and polyfluoroalkyl substances (PFAS) are widespread persistent organic pollutants and endocrine disruptors. High doses of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) exposure can cause pregnancy loss and infant deaths in animals, but the associations between PFAS exposures and risk of miscarriage in humans are not well studied.

METHODS: Using a case–control study nested within the Danish National Birth Cohort (DNBC, 1996–2002), we compared 220 pregnancies ending in miscarriage during weeks 12–22 of gestation, with 218 pregnancies resulting in live births. Levels of seven types of PFAS [PFOS, PFOA, perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluorooctanesulfonic acid (PFOSA)] were measured in maternal plasma collected in early gestation (mean gestational week 8). We estimated the odds ratios (ORs) and 95% confidence intervals (CIs) for miscarriage and each PFAS as a continuous variable or in quartiles, controlling for maternal age, parity, socio-occupational status, smoking and alcohol intake, gestational week of blood sampling, and maternal history of miscarriage. Stratification by parity and PFAS mixture analyses using weighted quantile sum (WQS) regression were also conducted.

RESULTS: We observed a monotonic increase in odds for miscarriage associated with increasing PFOA and PFHpS levels. The ORs comparing the highest PFOA or PFHpS quartile to the lowest were 2.2 (95% CI: 1.2, 3.9) and 1.8 (95% CI: 1.0, 3.2). The ORs were also elevated for the second or third quartile of PFHxS or PFOS, but no consistent exposure–outcome pattern emerged. An interquartile range (IQR) increment in the WQS index of seven PFAS was associated with 64% higher odds for miscarriage (95% CI: 1.15, 2.34). The associations were stronger in parous women, while findings were inconsistent among nulliparous women.

CONCLUSION: Maternal exposures to higher levels of PFOA, PFHpS, and PFAS mixtures were associated with the risk of miscarriage and particularly among parous women. Larger replication studies among nulliparous women are needed to allay concerns about confounding by reproductive history. https://doi.org/10.1289/EHP6202

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic fluorine-containing chemicals that are widespread and persistent in the environment (Houde et al. 2006; Lau et al. 2007). PFAS have been applied in a variety of commercial products since the 1940s, such as in the treatment of paper, clothing, carpets, food packing material, and kitchenware (Houde et al. 2006). Dietary exposure and contaminations from drinking water, food packaging material, indoor air, and household environments are likely the major exposure routes in humans (D'eon and Mabury 2011). The most frequently detected PFAS have estimated biological half-lives in humans of about 3 to 5 y or longer (Olsen et al. 2007). Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were the two most commonly used PFAS that have now been gradually phased out in production, but these compounds are still detectable globally (Bjerregaard-Olesen et al.

2016; Calafat et al. 2019; Kato et al. 2011). Meanwhile, human exposures to other types of PFAS, such as perfluorononanoic acid (PFNA), have been reported to be increasing (Bjerregaard-Olesen et al. 2016; Kato et al. 2011). Moreover, newer types of fluorinated compounds, such as GenX (also named PFPrOPrA or HFPO–DA) (Gebbink et al. 2017), designed as substitutes for PFOA in manufacturing processes, have recently been detected in the biota and drinking water sources (Gebbink et al. 2017; Sun et al. 2016). Continuing research efforts to evaluate potential adverse health effects resulting from human exposures to these synthetic fluorinated compounds are still needed.

Experimental studies have demonstrated a rather strong developmental toxicity of PFOA and PFOS; for example, exposure to high doses of PFOS and PFOA can cause pregnancy loss, infant mortality, birth defects, and impaired fetal growth in mice (Lau et al. 2006; Luebker et al. 2005). In humans, considerable amounts of PFAS from the mothers can cross the placental barrier and accumulate in the fetus (Fei et al. 2007; Manzano-Salgado et al. 2015). Numerous epidemiological studies have suggested that prenatal PFAS exposure might affect fetal growth (Bjerregaard-Olesen et al. 2019; Fei et al. 2008; Liew et al. 2018a) and increase risk for preterm birth (Meng et al. 2018).

Miscarriage, defined as fetal loss prior to 20 or 22 wk of gestation, is common and estimated to affect about 10–20% of all clinically recognized pregnancies (Nybo Andersen et al. 2000; Wilcox et al. 1988). Epidemiological evidence regarding the possible link between PFAS exposure and miscarriage is sparse. The C8 Health Project that surveyed a community highly exposed to PFOA from contaminated drinking water reported no associations for the history of miscarriage and stillbirths according to the geospatial modeled PFOA and PFOS levels (Darrow et al. 2013; Savitz et al.

2012; Stein et al. 2009). A more recent report in the C8 Health Project, which employed a prospective design and assessed serum PFOA and PFOS concentrations in 1,129 women during 2005–2006 and their self-reported pregnancy outcomes between 2008 and 2011, found 20–30% higher odds for miscarriage per log ng/mL increase of PFOS level in the women prior to pregnancy, while no association was found for PFOA (Darrow et al. 2014). Another small case–control study, which included 56 cases of miscarriage selected from a pregnancy cohort among women who resided in the municipality of Odense, Denmark, during 2010–2012, estimated an unexpectedly high 2- to 16-fold odds for miscarriage in the highest tertile of two types of PFAS [PFNA and perfluorodecanoic acid (PFDA)], measured in first-trimester maternal serum, while no association was found for PFOA and PFOS (Jensen et al. 2015).

We conducted a nested case–control study using maternal blood samples collected in the Danish National Birth Cohort (DNBC) and evaluated the associations between prenatal exposure to seven types of PFAS and the risk of miscarriage.

Materials and Methods

Cases and Controls

The DNBC enrolled 100,413 pregnancies at the first antenatal visit (weeks 6 to 12) through general practitioners from 1996–2002 in all regions of Denmark (Olsen et al. 2001). Pregnant women who completed the first telephone interview invited approximately at week 12 of gestation and with a stored prenatal blood sample available for PFAS analyses were eligible for this study. All participating women enrolled in the DNBC intended to carry their pregnancies to term. Information on miscarriage and the gestational age of event was obtained from the Danish National Hospital Discharge Register (Andersen et al. 1999; Bech et al. 2005). We randomly selected 220 cases among 760 eligible pregnancies ending in miscarriage during weeks 12–22. We also randomly selected 220 controls from 80,375 eligible pregnancies ending in singleton live births registered in the Danish Medical Birth Register (Bliddal et al. 2018). The sample size was predefined and limited by the study cost to conduct PFAS measurement. Two control samples failed the PFAS extraction process; thus, the final sample size for statistical analysis was 220 cases and 218 controls.

The research protocol for this study was approved by the Danish data inspectorate (2015-57-0002) and the Danish ethical review committee (1-10-72-134-17).

Per- and Polyfluoroalkyl Substances Measurements

Details of the analytic methods for PFAS have been described previously (Liew et al. 2014, 2015b). Briefly, the maternal blood samples in the DNBC were collected at the first antenatal visit at the general practitioner (around gestational week 8 on average for this sample) and were sent by regular mail to the biobank located at Statens Serum Institut in Copenhagen to be separated and stored in freezers at $-20°C$ or $-80°C$ (Kato et al. 2013). We requested 0.1 mL of the stored maternal plasma from the selected cases and controls, and these samples were sorted in random order before transferring to the Department of Environmental Science at Aarhus University for PFAS analyses. The solid-phase extraction technique was used for sample extraction and purification. PFAS concentrations were measured by liquid chromatography–tandem mass spectrometry. We measured 16 different PFAS, and the full panel for the lower limit of quantitation (LLOQ) and the distributions of all PFAS are presented in Table S1. In this study, we focused on seven types of PFAS that were quantified for at least 75% of the cases and controls samples, including PFOS, PFOA,

perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), PFDA, perfluorooctanesulfonic acid (PFOSA), and PFNA. Table S2 displays the comparison of the PFAS values in our samples with those reported for participants of the U.S. National Health and Nutrition Examination Survey (NHANES) 1999–2000 (Calafat et al. 2007) and the two other previous studies that had investigated the associations between PFAS exposures and risk for miscarriage (Darrow et al. 2014; Jensen et al. 2015).

Statistical Analysis

We used unconditional logistic regression model to estimate odds ratio (OR) and 95% confidence interval (CI) for prenatal PFAS exposure and miscarriage. The PFAS levels were first analyzed as continuous values after log transformation (base 2) to reduce the influence of outliers, and the exposure contrast represents per doubling increase of the PFAS concentration (ng/mL). Next, we categorized PFAS values into quartiles according to the distribution among controls using the lowest quartile as the reference group. To evaluate linear dose–response, trend tests were performed using median values of PFAS in each quartile as a continuous variable. Moreover, we fitted a generalized additive model including a spline term for the continuous PFAS value (ng/mL) with 3 degrees of freedom to evaluate potential nonlinear relation and to visualize exposure–outcome response.

PFAS levels below the LLOQ and missing covariate values were replaced using the multiple imputation procedure proc mi in SAS (version 9.2; SAS Institute Inc.) including seven PFAS, the case or control status, and all potential confounders considered in this study assuming variables were missing at random (Lubin et al. 2004). Less than 4% of participants had at least one missing value for covariate. Ten simulated complete data sets were generated via imputation, and we employed the analytical procedure proc mianalyze in SAS to combine the results (Yuan 2001).

Potential confounders were selected *a priori* according to directed acyclic graphs (see Figure S1). Firstly, we controlled for gestational week of blood sampling as a categorical variable in strata of 4–6, 7–8, 9–10, and >11 wk in all analyses. The gestational week of blood sampling is important because it was conceived as the study entry time for the pregnancy cohort on fetal loss. The risk of miscarriage is the highest in early pregnancy and declines with increasing gestational age (Macklon et al. 2002). Moreover, we included maternal age (<30, ≥30 y), parity (nulliparous, parous), parental socio-occupational status (high vs. medium/low), and maternal first-trimester smoking (yes or no) or alcohol intake (yes or no) in the main model. Information on estimated gestational week, maternal age, and parity were obtained from the Danish medical birth register, while maternal smoking, alcohol intake, and socio-occupational status was collected from the first telephone interview. Parental socio-occupational status was derived from the reported education and occupation of the mothers and the fathers (Bech et al. 2005; Liew et al. 2018b). High level included participants if either of the couple was working in high-grade professional that required longer-term education, medium level included medium-term education and/or skilled worker, and low level included unskilled workers, unemployment, or on financial assistance. Very few participants in the DNBC (<4%) were classified in the low level; thus, this category was combined with the medium group in analyses. To avoid sparse data bias (Greenland et al. 2016), most of the main potential confounders were adjusted as binary response given a range of variables considered that were also interrelated. Adjustment for finer categories of maternal age (<25, 25–35, or ≥35 y) or parity (0, 1, or ≥2) were evaluated in sensitivity analyses.

In addition, we adjusted for maternal history of miscarriage based on information obtained from the Danish National Hospital Discharge Register to further account for possible genetics, diseases,

and/or other familial factors that might affect reoccurring miscarriages (Rai and Regan 2006). Furthermore, we calculated the time gap (in years) since last pregnancy for women who have had a previous pregnancy, regardless of the birth outcome. Time since last pregnancy was expected to correlate with maternal PFAS level in the subsequent pregnancy due to reaccumulation of the chemicals after the preceding pregnancy event (Bach et al. 2018), and interpregnancy interval could be associated with pregnancy outcomes (Love et al. 2010). Prior breastfeeding could also reduce the body burden of PFAS in parous women (Lauritzen et al. 2016). We did not have information on prior breastfeeding. Nonetheless, since the prevalence of breastfeeding was high in this cohort (Fei et al. 2010), we assumed that women whose last pregnancy outcome was a live birth most likely breastfed their babies. We therefore used a model additionally adjusted for the outcome of last pregnancy (no pregnancy history, live birth, unsuccessful pregnancy including miscarriage, stillbirth, induced abortion, or ectopic pregnancy) and the time since last pregnancy (no pregnancy history, <1 y, 1 to <2 y, 2 to <3 y, 3 to <6 y, and >6 y) while leaving out parity and history of miscarriage to avoid overadjustment or collinearity. Finally, in sensitivity analyses, we adjusted for additional potential confounders including pregnancy year, maternal prepregnancy body mass index (BMI), and estimated season of conception. We also excluded women reporting having hypertension, metabolic disorders, or chronic kidney diseases during pregnancy in the first telephone interview (n = 18 cases and 14 controls).

We conducted stratified analyses for parity, maternal age, and socio-occupational status because these factors had previously been suggested to be associated with the risk for miscarriage in the DNBC (Feodor Nilsson et al. 2014; Norsker et al. 2012) and they could potentially modify the effect estimates of PFAS exposures (Chang et al. 2016; Liew et al. 2018a). Tests of heterogeneity were performed by assessing the p-value of the interaction term for each PFAS and potential modifying factors in the regression models.

The Pearson correlation matrix for the seven PFAS in controls was estimated (Table S3). To disentangle the possible effect of each of the PFAS (Braun et al. 2016), we constructed coadjusted pollutants models: model 1 coadjusted for PFOS and PFOA (the most widespread PFAS), model 2 included the four most studied PFAS (PFOS, PFOA, PFHxS, and PFNA) (Calafat et al. 2007; Darrow 2014; Jensen 2015), and, finally, model 3 coadjusted for all seven types of PFAS. Moreover, we used the weighted quantile sum (WQS) regression to estimate a joint exposure effect of the PFAS mixture on miscarriage risk (Carrico et al. 2015; Cluett et al. 2019). A WQS index was created using all seven types of PFAS, with each PFAS assigned a weight that reflected the strength of that PFAS and outcome association and the collinearity between that PFAS and other types of PFAS in the mixture (Carrico et al. 2015). We constrained the sum of weights to 1.0 to incorporate each mixture component into a single-effect estimate, and we also constrained each mixture component effect to be positive based on findings from the individual PFAS model. The WQS index was scaled for an interquartile range (IQR) increase to improve interpretability.

Data analyses were performed using SAS (version 9.2; SAS Institute Inc.) and the gWQS package in R (version 3.6; R Development Core Team).

Results

Table 1 presents the characteristics of the case and control women. The case women were more likely to be smokers, parous, and have had a history of miscarriage compared to the controls. Among the seven PFAS, the proportions above the LLOQ in the control samples were 100% PFOS, 100% PFOA, 100% PFHxS, 99.5% PFHpS, 99.1% PFDA, 89.0% PFOSA, and 88.5% PFNA, while those for

Table 1. Study characteristics of cases and controls.

	Case (n = 220)		Control (n = 218)	
Characteristic	n	%	n	%
Maternal age (y)				
<30	93	42.3	95	43.5
≥30	127	57.7	123	56.4
Socio-occupational status				
High	146	66.4	138	63.3
Medium/low	74	33.6	80	36.7
Parity				
Nulliparous	101	45.9	98	45.0
Parous	119	54.1	120	55.0
Smoking during first trimester				
No	153	69.5	166	76.2
Yes	67	30.5	52	23.8
Alcohol drinking during first trimester				
No	125	56.8	118	54.1
Yes	95	43.2	98	45.0
Missing	0	0	2	0.9
Maternal history of miscarriage				
No	179	81.4	182	83.5
Yes	41	18.6	36	16.5
The outcome of last pregnancy				
No pregnancy history	73	33.2	77	35.3
Live birth	92	41.8	96	44.1
Unsuccessful pregnancies[a]	55	25.0	45	20.6
Time since last pregnancy (y)				
No pregnancy history	73	33.2	77	35.3
Less than 1	25	11.4	16	7.3
1 to <2	17	7.7	31	14.2
2 to <3	30	13.6	39	17.9
3 to <6	46	20.9	43	19.7
>6	29	13.2	12	5.5
Gestational week of blood sampling				
Mean (SD)	7.4	2.4	8.7	2.3
4–6	71	32.3	34	15.6
7–8	67	30.4	84	38.5
9–10	57	25.9	59	27.1
>11	18	8.2	41	18.8
Missing	7	3.2	0	0

Note: SD, standard deviation.
[a]Defined as having a miscarriage, stillbirth, induced abortion, or ectopic pregnancy.

the case samples were 100% PFOS, 100% PFOA, 100% PFHxS, 99.5% PFHpS, 99.1% PFDA, 90.9% PFOSA, and 89.1% PFNA. The medians and IQRs of the seven maternal plasma PFAS concentrations in the cases and control samples are presented in Table S1. We found that a doubling increase of PFOA was associated with 40% elevated odds for miscarriage (OR = 1.4; 95% CI: 1.0, 1.9) after adjusting for main confounders (Table 2). The effect estimates changed minimally in models that additionally controlled for the woman's history of miscarriage, her last pregnancy outcome, and the time gap since last pregnancy. The estimated ORs were also elevated for PFHpS or PFOS, but the CIs included the null. No associations were found for other types of PFAS.

In analyses of PFAS quartiles, the estimated odds for miscarriage were more than doubled for the highest PFOA quartile (OR = 2.2; 95% CI: 1.2, 3.9) and had an 80% increase for the highest PFHpS quartile (OR = 1.8; 95% CI: 1.0, 3.2) compared with the reference (Table 3). A monotonic increase in ORs according to PFAS quartile was also observed for these two compounds (p-values for trend ≤0.01 for PFOA and 0.03 for PFHpS) (Table 3). Further adjustment for the last pregnancy outcome and the time gap since last pregnancy slightly attenuated the association for PFOA but did not change the estimates for other PFAS. The estimated ORs seemed also to be elevated for the third and fourth quartiles of PFOS and the second or third quartiles of PFHxS and PFOSA, but there was no clear linear response (Table 3).

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for miscarriage according to per-doubling increase of PFAS (ng/mL) in early pregnancy.

Prenatal PFAS	Crude OR (95% CI)	Model A[a] Adjusted OR (95% CI)	Model B[b] Adjusted OR (95% CI)	Model C[c] Adjusted OR (95% CI)
PFOS	1.2 (0.9, 1.7)	1.2 (0.9, 1.8)	1.3 (0.9, 1.8)	1.2 (0.8, 1.7)
PFOA	1.2 (0.9, 1.7)	1.4 (1.0, 1.9)	1.4 (1.0, 1.9)	1.3 (0.9, 1.8)
PFHxS	0.9 (0.7, 1.2)	0.9 (0.7, 1.3)	0.9 (0.7, 1.3)	0.9 (0.7, 1.3)
PFHpS	1.3 (0.9, 1.8)	1.3 (0.9, 1.9)	1.3 (0.9, 1.9)	1.3 (0.9, 1.8)
PFDA	1.1 (0.8, 1.6)	1.1 (0.8, 1.7)	1.1 (0.8, 1.7)	1.1 (0.7, 1.6)
PFOSA	1.0 (0.9, 1.2)	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)
PFNA	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	1.0 (0.8, 1.4)	1.0 (0.8, 1.3)

Note: We analyzed miscarriage cases and 218 controls. PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFOSA, perfluorooctanesulfonic acid.
[a]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, and parity.
[b]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, parity, and maternal history of miscarriage.
[c]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, the outcome of last pregnancy, and time gap since last pregnancy.

Table 3. Odds ratios (ORs) and 95% confidence interval (CIs) for miscarriage according to PFAS quartiles in early pregnancy.

PFAS quartile[a]	Cases/controls	Model A[b] Adjusted OR (95% CI)	Model B[c] Adjusted OR (95% CI)	Model C[d] Adjusted OR (95% CI)
PFOS				
Q1	47/55	Ref	Ref	Ref
Q2	49/54	1.1 (0.6, 1.9)	1.1 (0.6, 1.9)	1.1 (0.6, 2.0)
Q3	61/55	1.3 (0.8, 2.4)	1.4 (0.8, 2.4)	1.4 (0.8, 2.5)
Q4	63/54	1.4 (0.8, 2.4)	1.4 (0.8, 2.5)	1.3 (0.7, 2.3)
p for trend	220/218	0.21	0.21	0.35
PFOA				
Q1	46/55	Ref	Ref	Ref
Q2	44/54	1.0 (0.5, 1.8)	1.0 (0.5, 1.8)	0.9 (0.5, 1.7)
Q3	53/55	1.4 (0.8, 2.6)	1.4 (0.8, 2.6)	1.4 (0.7, 2.5)
Q4	77/54	2.2 (1.2, 3.9)	2.2 (1.2, 3.9)	1.9 (1.0, 3.6)
p for trend	220/218	<0.01	<0.01	0.02
PFHxS				
Q1	48/56	Ref	Ref	Ref
Q2	66/54	1.3 (0.8, 2.3)	1.4 (0.8, 2.4)	1.3 (0.7, 2.3)
Q3	60/54	1.3 (0.7, 2.3)	1.3 (0.7, 2.3)	1.3 (0.7, 2.2)
Q4	46/54	0.9 (0.5, 1.7)	0.9 (0.5, 1.7)	0.9 (0.5, 1.7)
p for trend	220/218	0.68	0.67	0.69
PFHpS				
Q1	46/62	Ref	Ref	Ref
Q2	49/50	1.3 (0.7, 2.3)	1.3 (0.7, 2.3)	1.3 (0.7, 2.3)
Q3	56/52	1.5 (0.9, 2.7)	1.6 (0.9, 2.8)	1.6 (0.9, 2.9)
Q4	69/54	1.8 (1.0, 3.2)	1.8 (1.0, 3.3)	1.8 (1.0, 3.2)
p for trend	220/218	0.03	0.03	0.04
PFDA				
Q1	45/50	Ref	Ref	Ref
Q2	60/58	1.0 (0.6, 1.7)	1.0 (0.6, 1.7)	1.0 (0.6, 1.8)
Q3	50/43	1.1 (0.7, 1.9)	1.1 (0.7, 1.9)	1.1 (0.6, 1.9)
Q4	65/67	1.3 (0.7, 2.2)	1.3 (0.7, 2.3)	1.2 (0.7, 2.1)
p for trend	220/218	0.35	0.34	0.49
PFOSA				
Q1	44/55	Ref	Ref	Ref
Q2	64/54	1.5 (0.9, 2.7)	1.5 (0.9, 2.6)	1.5 (0.8, 2.7)
Q3	61/55	1.5 (0.8, 2.6)	1.5 (0.8, 2.6)	1.5 (0.9, 2.7)
Q4	51/54	1.3 (0.7, 2.3)	1.3 (0.7, 2.3)	1.3 (0.7, 2.4)
p for trend	220/218	0.78	0.80	0.66
PFNA				
Q1	62/59	Ref	Ref	Ref
Q2	46/55	0.9 (0.5, 1.5)	0.8 (0.5, 1.5)	0.8 (0.5, 1.5)
Q3	61/56	1.1 (0.6, 1.8)	1.0 (0.6, 1.8)	1.0 (0.5, 1.7)
Q4	51/48	1.0 (0.6, 1.8)	1.1 (0.6, 1.8)	1.0 (0.6, 1.8)
p for trend	220/218	0.72	0.69	0.82

Note: PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFOSA, perfluorooctanesulfonic acid; Ref, Reference.
[a]p for trend was fitted using the median value of PFAS in each quartile as a continuous variable.
[b]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, and parity.
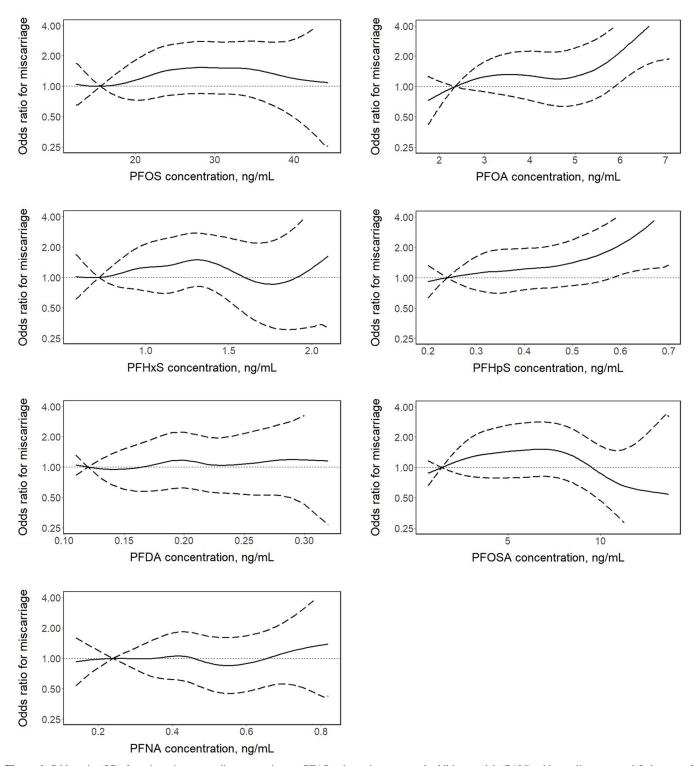[c]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, parity, and maternal history of miscarriage.
[d]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, the outcome of last pregnancy, and time gap since last pregnancy.

Figure 1. Odds ratio (OR) for miscarriage according to continuous PFAS value using a general additive model (GAM) with a spline term and 3 degrees of freedom. The 15th percentile of each PFAS level was used as the reference, and the graph plotted the exposure range from the 5th to 95th percentiles for each PFAS. The dashed lines represent 95% confidence intervals. Model adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, and parity. Note: PFDA, perfluorodecanoic acid; PFHpS, perfluoro-heptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFOSA, perfluorooctanesulfonic acid.

In the exposure spline model, we observed that the odds for miscarriage increased at higher levels of PFOA (starting from 3 ng/mL, and there was a sharper increase after 5 ng/mL) (Figure 1). The estimated OR was also gradually increased for a higher PFHpS level (>0.3 ng/mL). The estimated ORs were moderately elevated from a lower to the middle range for PFOS (20–40 ng/mL), PFHxS (1.0–1.4 ng/mL), and PFOSA (3–8 ng/mL), but the ORs decreased thereafter. The exposure and outcome trend for PFDA and PFNA were mostly flat in the spline model.

Table 4. Odds ratios (ORs) and 95% confidence interval (CIs) for miscarriage according to PFAS exposure in early pregnancy, stratified by parity.

	Nulliparous		Parous	
Prenatal PFAS[a]	Cases/controls	Adjusted OR[b] (95% CI)	Cases/controls	Adjusted OR[b] (95% CI)
PFOS				
Per doubling	101/98	0.9 (0.5, 1.5)	119/120	1.4 (0.8, 2.4)
Q1	25/25	Ref	26/34	Ref
Q2	25/25	1.0 (0.4, 2.6)	28/32	1.2 (0.5, 2.6)
Q3	28/22	1.1 (0.4, 2.8)	29/31	1.4 (0.6, 3.2)
Q4	23/26	0.7 (0.3, 1.8)	36/23	1.9 (0.8, 4.4)
p for trend	101/98	0.47	119/120	0.10
PFOA				
Per doubling	101/98	1.2 (0.6, 2.1)	119/120	1.2 (0.7, 2.0)
Q1	25/25	Ref	26/34	Ref
Q2	22/28	1.2 (0.5, 3.0)	26/35	0.8 (0.4, 1.8)
Q3	31/20	1.7 (0.7, 4.4)	28/32	1.0 (0.4, 2.2)
Q4	23/25	1.1 (0.4, 2.9)	39/19	2.4 (1.0, 5.8)
p for trend	101/98	0.70	119/120	0.04
PFHxS				
Per doubling	101/98	0.9 (0.5, 1.7)	119/120	0.9 (0.6, 1.3)
Q1	23/27	Ref	29/32	Ref
Q2	24/26	1.3 (0.5, 3.4)	32/27	0.9 (0.4, 2.1)
Q3	31/19	2.4 (0.9, 5.9)	31/30	0.9 (0.4, 2.0)
Q4	23/26	0.9 (0.4, 2.3)	27/31	1.0 (0.4, 2.1)
p for trend	101/98	0.98	119/120	0.93
PFHpS				
Per doubling	101/98	1.0 (0.6, 1.7)	119/120	1.5 (0.9, 2.7)
Q1	23/27	Ref	26/37	Ref
Q2	26/27	0.9 (0.3, 2.1)	29/31	1.2 (0.6, 2.7)
Q3	27/20	1.4 (0.6, 3.6)	31/31	1.5 (0.7, 3.3)
Q4	25/24	1.3 (0.5, 3.3)	33/21	2.4 (1.0, 5.6)
p for trend	101/98	0.39	119/120	0.03
PFDA				
Per doubling	101/98	0.8 (0.4, 1.6)	119/120	1.2 (0.7, 2.2)
Q1	29/25	Ref	36/42	Ref
Q2	20/27	0.6 (0.2, 1.5)	25/23	1.4 (0.6, 3.0)
Q3	29/23	1.0 (0.4, 2.3)	27/27	1.2 (0.5, 2.5)
Q4	23/23	0.8 (0.3, 2.1)	31/28	1.3 (0.6, 2.7)
p for trend	101/98	0.95	119/120	0.63
PFOSA				
Per doubling	101/98	1.1 (0.8, 1.4)	119/120	1.1 (0.9, 1.4)
Q1	21/29	Ref	28/32	Ref
Q2	29/21	1.9 (0.8, 4.8)	29/31	1.0 (0.5, 2.2)
Q3	27/23	1.5 (0.6, 3.7)	33/27	1.9 (0.8, 4.4)
Q4	24/25	1.5 (0.6, 3.6)	29/30	1.2 (0.5, 2.7)
p for trend	101/98	0.66	119/120	0.63
PFNA				
Per doubling	101/98	0.8 (0.5, 1.2)	119/120	1.2 (0.8, 1.7)
Q1	25/25	Ref	32/30	Ref
Q2	25/25	0.9 (0.4, 2.3)	30/33	1.0 (0.4, 2.1)
Q3	29/21	1.1 (0.4, 2.7)	27/32	0.7 (0.3, 1.6)
Q4	22/27	0.7 (0.3, 1.7)	30/25	1.1 (0.5, 2.4)
p for trend	101/98	0.45	119/120	0.84

Note: PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFOSA, perfluorooctanesulfonic acid; Ref, Reference.
[a]PFAS quartiles were created separately for parous and nulliparous women based on PFAS distributions in controls. *p* for trend was fitted using the median value of PFAS in each quartile as a continuous variable.
[b]Adjusted for maternal age, parental socio-occupational status, maternal smoking in the first trimester, maternal alcohol intake in the first trimester, gestational week of blood sampling, and time gap since last pregnancy.

In multiple PFAS adjustments, the positive effect sizes between PFOA or PFHpS and miscarriage remained unchanged, even in the model coadjusted for seven types of PFAS, while the effect estimates for other types of PFAS were null (Table S4). In mixture analyses, the WQS index of seven PFAS was positively associated with miscarriage [each IQR increment in the WQS index was associated with a 64% higher odds for miscarriage (95% CI: 1.15, 2.34)]. Within the mixture, PFOA had the highest weight (60%), followed by PFHpS (17%) and PFDA (11%), and the weight was low for PFOS (5%), PFOSA (3%), PFHxS (2%), and PFNA (2%).

Findings remained similar in models further adjusting for pregnancy year, maternal prepregnancy BMI, and season of conception (Tables S5 and S6). Results also did not change when maternal age and parity were classified in finer categories (Table S6) or when women with hypertension, metabolic disorders, or chronic kidney diseases in pregnancy were excluded in analyses (Table S7).

Stratified analyses by parity suggested that the positive effect estimates between increasing PFOS, PFOA or PFHpS and miscarriage were more consistent in parous women, but the *p*-values for interaction were greater than 0.10 for all seven PFAS. For nulliparous women, no linear dose–response association was found for any of the PFAS, but the estimates were elevated for the second quartile and the third quartile for several PFAS, including PFOA, PFHxS, and PFOSA compared with the lowest quartile (Table 4). The positive effect estimates between continuous PFOS, PFOA, or PFHpS and miscarriage were also more

apparent among younger women and those from lower socio-occupational status (Table S8), but all *p*-values for interaction were greater than 0.23 for all PFAS.

Discussion

In this case–control study nested within the DNBC, we found that exposures to two types of PFAS, PFOA and PFHpS, were most consistently associated with risk for miscarriage. Although the last pregnancy outcome and time gap since last pregnancy has been controlled for in analyses, the effect estimates seem to be stronger in parous women, which raised concerns of possible residual confounding from women's reproductive history that has been suggested in the studies of PFAS exposure and fertility among pregnant women (Bach et al. 2018). Chance error is also possible with multiple types of PFAS examined and for results in subgroup analyses. Future study with a larger sample size for nulliparous women could help to rule out the influence of random errors and the concern for confounding by pregnancy history.

Losing a pregnancy, especially in later pregnancy, may have detrimental effects on the mental health and general well-being of the couples (Lee and Slade 1996). The risk of miscarriage is greater for older parents and among women with a previous miscarriage (Magnus et al. 2019). Other suggested modifiable risk factors for miscarriage include maternal diseases, infections, obesity, exposures to tobacco smoke, and drug or alcohol use (Boots and Stephenson 2011; Feodor Nilsson et al. 2014; Giakoumelou et al. 2016). A recent study conducted in the DNBC estimated nearly a quarter of the miscarriages might be preventable by intervening on these recognized sociodemographic and behavioral risk factors before or during pregnancy (Feodor Nilsson et al. 2014), but risks for the majority of the miscarriage are still unexplained. There is a need to search for additional modifiable causes for miscarriage.

Exposures to widespread endocrine-disrupting chemicals that could interfere with the maternal and fetal endogenous hormone action might play a role in affecting risks for miscarriage (Krieg et al. 2016). Maternal exposures to other persistent and synthetic chemicals such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), or dioxin have been linked to increased risk of pregnancy loss in a few epidemiological studies (Korrick et al. 2001; Krieg et al. 2016; Meeker et al. 2011; Pan et al. 2015; Venners et al. 2005). Research evidence has also consistently suggested that PFAS could interfere with immunological, metabolic, and hormonal function in pregnancy (Ballesteros et al. 2017; Matilla-Santander et al. 2017). Studies that investigated PFAS exposures on subfecundity reported mixed findings in part due to methodological challenges (Bach et al. 2018, 2015), especially that an association found among parous women could reflect reverse causation bias because a longer interpregnancy interval of a prior birth is correlated with the reaccumulation of PFAS (Whitworth et al. 2012). A more recent cohort that followed 501 couples daily from preconception through 7 postconception weeks reported no associations for seven types of PFAS and incidence of early pregnancy loss (Buck Louis et al. 2016).

The association between PFAS and miscarriage has only been investigated in two populations, including the C8 Health Project (Darrow et al. 2013, 2014; Savitz et al. 2012; Stein et al. 2009) and another smaller Danish cohort (Jensen et al. 2015). The exposure level of PFOA in the C8 Health Project was about three to four times higher compared with the general population, which might limit the generalizability of findings. The Danish study conducted in the municipality of Odense during 2010–2012 reported 200–1,600% increased odds in the highest tertile of first-trimester serum level of PFNA and PFDA (Jensen et al. 2015). These effect estimates seemed to be unrealistically high and raised concerns of sparse data bias. Our study used larger samples

to obtain more precise statistical estimates and found no associations for PFNA and PFDA but moderate positive associations of PFOA and PFHpS with miscarriage risk. The recruitment of pregnant women in the DNBC was nationwide, but the enrollment was conducted 10–15 y before the Odense study, and since then, PFOS and PFOA levels in Denmark have declined, while some increases in PFNA and PFHxS level were observed. These changes in PFAS exposure levels in Denmark might explain the discrepancies of findings from the two Danish cohorts.

PFAS are persistent, and our measure in early pregnancy is likely also reflecting cumulative exposures over the years; thus, the results from analyses adjusting for maternal history of miscarriage, the last pregnancy event, and also time gap between pregnancy should be interpreted with caution because these factors could also lie between the causal pathways between PFAS and risk for miscarriage (Howards et al. 2012). Adjusting for these factors slightly attenuated the effect estimates for some PFAS but did not change the overall findings. Maternal parity is a strong predictor of maternal body burden of PFAS in the subsequent pregnancy for parous women because of transplacental transfer of PFAS (Fei et al. 2007; Verner et al. 2016). Although PFAS are lipophobic and detected in lower concentrations in breastmilk compared with other lipophilic persistent pollutants such as PCBs and dioxins (von Ehrenstein et al. 2009), duration of breastfeeding contributes to infancy exposure and thus also the excretion of PFAS in the mother (Verner et al. 2016). While the duration of prior breastfeeding for parous women at enrollment was unknown, the median duration of any breast-feeding and full breastfeeding for all mothers in the cohort was 7.5 months and 4.0 months, respectively (Kirkegaard et al. 2018). Interpregnancy interval is strongly correlated with the measures of time to pregnancy and fertility in multigravida women, but the associations with risk for miscarriage is unclear (Kangatharan et al. 2017; Love et al. 2010). Unlike the C8 Health Project, which reported a stronger association between PFOS and miscarriage among nulliparous women (Darrow et al. 2014), the associations found between PFAS and risk for miscarriage appeared to be more consistent among parous women in our sample. A future study focusing on miscarriage risk in first pregnancy could help to alleviate concerns about uncontrolled confounding by reproductive history. Moreover, a potential modifying effect for younger maternal age, timing of exposure, and lower socio-occupational status (Buekers et al. 2018) should also be followed up using a larger sample.

The critical time window of exposure in relation to miscarriage risk is unknown. The average gestational week of blood sampling for our study is similar to another Danish study (Jensen et al. 2015). Reports from the C8 study did not have a clear timing because the exposure was either extrapolated based on a geospatial model (Darrow et al. 2013; Savitz et al. 2012; Stein et al. 2009) or the study correlated serum PFAS in reproductive age women and their miscarriage events 3–5 y after the exposure assessments (Darrow et al. 2014). The persistent PFAS have been shown to be highly correlated using repeated blood samples in the first and the second trimesters collected in the DNBC (Fei et al. 2007). However, effect measure modification by gestational week of sampling is also possible and has recently been suggested for the thyroid hormone changes in early pregnancy (Inoue et al. 2019). Our stratified analyses suggested a stronger effect estimate for samples collected after gestational week 8, but the stratified analyses were imprecise and need to be reevaluated in future research.

Our analyses did not account for other hemodynamic factors such as albumin and glomerular filtration rate (GFR) that could have affected the PFAS measure during pregnancy (Verner et al. 2015). However, confounding by GFR has been suggested to be more important if maternal blood samples were collected later in

pregnancy. Excluding women with hypertensive, metabolic, and kidney diseases in our analyses did not change the findings. We also cannot rule out residual confounding from other unmeasured or unknown confounders, such as dietary habits, lifestyles, and household factors (Halldorsson et al. 2008). Moreover, we do not have measures of other persistent organic pollutants (POPs). Studies have reported that PFAS are only weakly correlated with other classes of POPs (Fisher et al. 2016); thus, potential confounding from these other persistent chemicals are expected to be minimal. However, if these different types of chemicals affect similar biological pathways, the joint exposure effects could be larger than assessing each of the exposure separately (Braun et al. 2016).

Our findings might be susceptible to selection bias due to fetal survival because the cases and controls sampling are conditioned on fetal survival up to gestational week 12 of pregnancies (Liew et al. 2015a; Lisonkova and Slade 2015). However, a U.S. study of 501 couples recruited preconception and followed daily through 7 postconception weeks reported no strong association between seven types of PFAS and 98 cases of early pregnancy loss (Louis et al. 2016). We did not study pregnancies ending in early pregnancy losses because of possible incomplete registration of these outcomes in the DNBC. We might expect a bias toward the null if high PFAS exposure disproportionally removes fetuses most susceptible to a loss at an earlier time point in pregnancy that otherwise would have occurred later, but the bias could also go in a different direction.

Our study has a number of strengths. First, the cases and controls were selected from the DNBC that enrolled women from early pregnancy, allowing prospective assessment of PFAS exposure using stored maternal blood samples. Secondly, the outcome was ascertained from records in the Danish National Hospital Discharge Register. A validation study has evaluated randomly selected records of miscarriage in the register from 1980 to 2008 and reported a high positive predictive value of 97.4% (95% CI: 92.7, 99.5) (Lohse et al. 2010). Finally, we were able to control for a wide range of potential confounders, including the multiple PFAS adjustments, and PFAS mixture analyses suggested robust results for PFOA and PFHpS.

In summary, we found that higher maternal plasma levels of two types of PFAS, PFOA and PFHpS, were most consistently associated with an increased risk for miscarriage during weeks 12–22 of gestations among parous women. The strongest effect estimates were about 2-fold increased for the highest PFOA or PFHpS quartile, which were concerning but were not as unrealistically high as the 16 times higher excess risk suggested by another smaller study in Denmark (Jensen et al. 2015). Mechanistic studies are needed to elucidate the possible biological mechanisms that explain these associations. Moreover, larger epidemiological studies are needed to replicate our findings, evaluate the possible threshold or dose-dependent effects of PFAS exposure on pregnancy loss, and address confounding by pregnancy history.

Acknowledgments

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